

Reply

To the Editor:

The letter from Drs McKenzie and Szepletowski in connection with our article (Hu *et al*, 2000) extended our observation and discussed the probable function of LIF in skin. The letter is helpful in understanding the cytokine network in skin. We agree with most of their viewpoints.

In the letter, however, they wrote: Hu *et al* "recorded no or negligible LIF immunoreactivity in disease control skin", whereas their group "always observed LIF immunoreactivity in normal skin". Quantitative comparison should be taken under the same conditions. In McKenzie's report (Paglis *et al*, 1996), the LIF immunostaining in normal skin seemed strong. But they used different immunohistochemical conditions from ours, by which the intensity of immunostain will be strongly affected. We used a different antibody (an affinity-purified polyclonal antibody against the LIF peptide, N-18, Santa Cruz, CA), a different immunoreactive condition (concentration of primary antibody 1 μ g per ml, incubated for 12 h), and a different section method (paraffin-embedded section). The normal skin samples they used were obtained from surgery patients, and transported in DMEM medium to the laboratory. Traumatic reaction might occur in this step, resulting in the elevation of cytokine concentrations (including LIF). On the contrary, we fixed all the biopsy samples immediately in formalin. Furthermore, the ethnic difference should also be considered as an effective factor.

In our article, we compared LIF immunoreactivity in biopsied skins between ALS and other neurodegenerative disease controls. For ethical reasons, we did not include any biopsied skin specimens

from normal subjects. We could not know how strong the LIF immunoreactivity was in normal skin, and whether immunoreactivity in ALS skin was just the same as that in normal skin. Nevertheless, we did not negate the LIF immunoreactivity in the skin of disease control. We also thought that a low level of LIF immunoreactivity in normal skin was reasonable. We emphasized that ALS patients expressed far more LIF in skin than the disease control subjects did. In our study, the immunoreactivities in the skins of ALS cases and controls were detected simultaneously under the same experimental conditions. The immunoreactive intensity was expressed as optical density (OD, arbitrary unit). The OD of ALS skins ranged from 9.0 to 1.9, while that of disease controls ranged from 1.8 to 0.4. Although the immunoreactive pattern in ALS with an OD of 1.9 was similar to that in disease-control with an OD of 1.8, the ALS group was significantly different from the disease control group statistically. Furthermore, we found that the OD in ALS showed a progressive increase in relation to duration of the illness ($r = 0.82$, $p < 0.01$), suggesting that OD in long duration patients should be higher than that in normal controls. The OD in ALS patients with a duration of 3.2 y was 9.0, while that of 0.4 y was only 1.8. We do not believe OD in normal skin could be as high as 9.0, unless they were proved by simultaneous and quantitative comparison.

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Limited Concordance Between "Oakmoss" and Colophony in Clinical Patch Testing

To the Editor

Recently, Lepoittevin *et al* (2000) reported on the detection of various resin acids in "treemoss" perfume extracts, some of them identical to those found in colophony and its oxidation products, respectively. Furthermore, a considerable contamination of "oakmoss" raw material, especially the material used by Trolab (Reinbek, Germany) for manufacturing "oakmoss" patch test material, with these resin acids was found (5.6% resin acids and 0.7% 7-oxo-dehydroabietic acid, a sensitizing oxidation product of colophony). Accordingly, the majority of their 17 patients sensitized to colophony not only reacted to "treemoss" ($n = 12$), but also to "oakmoss" by Trolab ($n = 9$), but rarely to "oakmoss" by Chemotechnique (Malmo, Sweden, $n = 2$) containing less than 0.4% wt/wt resin acids.

To put these challenging results pointing to a tremendous potential for misdiagnosis into the perspective of clinical patch

testing with "oakmoss", national surveillance data of the IVDK (<http://www.ivdk.gwdg.de>) collected between January 1992 and December 1999, in the 34 participating centers (see footnote 1) were analyzed. Test substances were supplied by Trolab, results based on readings at 72 h following the standards of the International Contact Dermatitis Research Group. In the above period, 67,306 patients were patch tested to colophony (20% in petrolatum), which is contained in the standard test series, with 1433 (2.1%) weakly positive ("+"), 1154 (1.7%) strongly positive ("++" or "+++") and 697 (1.0%) equivocal ("?") or, rarely, irritant reactions. At the same time, 12,823 patients were patch tested to "oakmoss" (1% in petrolatum, the same as used by Lepoittevin *et al*, 2000), which is included in the fragrances series and two other special series. These are tested in a more focussed way, often as a breakdown of the fragrance mix in case of a positive reaction. Nearly all patients (98.4%) were also tested with colophony. Results obtained in this subgroup ($n = 12,614$) are presented in the format of a 5×5 contingency table (**Table I**) showing the joint distribution of patch test reactions to both test substances. Altogether 27.0% of all persons allergic to colophony reacted positively ("+" to "+++") to "oakmoss", whereas only 5.5% positive reactions to "oakmoss" were found in the rest. This finding points to a

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Table I. Patch test results with colophony (20% pet.) and "oakmoss" (1% pet., both by Trolab) in 12614 patients between 1992 and 1999

Colophony	"Oakmoss"					Sum/(%) Colophony
	neg.	?/IR	+	++	+++	
neg.	10905	253	416	164	55	11793 (93.5%)
?/IR	134	17	19	5	2	177 (1.4%)
+	261	21	41	20	7	350 (2.8%)
++	130	22	45	20	0	217 (1.7%)
+++	34	2	15	18	8	77 (0.6%)
Sum/(%) "Oakmoss"	11464 (90.9%)	315 (2.5%)	536 (4.2%)	227 (1.8%)	72 (0.6%)	12614 (100.0%)

substantial degree of association between the two patch tests (relative risk: 4.89, 95% confidence interval (CI): 4.22–5.67). Interestingly, the proportion of "oakmoss" positive patients ("+" to "+++") increased from 5.4% in patients not reacting to colophony, to 14.7% in those reacting with erythema only ("?"), to 19.4% in those with a "+" reaction, to 30.0% in those with a "++" reaction, and to 53.3% in those with very strong ("+++") reactions to colophony (Cochran-Armitage trend test: $p < 0.0001$), see **Table I**. To further address the question of concordance, i.e., whether the two tests yield more or less identical results, we calculated Cohen's kappa (Cohen, 1960). This standard measure in such analyses quantifies the chance-corrected proportion of agreement between two discrete ratings and gives a more informative quantitative summarization of concordance than the crude proportion of agreement for skewed distributions of ratings as in this case (Fleiss, 1981). Although 87.1% of the patients showed identical patch test results for both tests, the kappa value is only 0.13 (95% CI: 0.11–0.15) indicating a very low degree of concordance beyond chance.

While the analytical results of Lepoittevin *et al* (2000) give some insight into the pitfalls in patch testing (with natural materials), we have some reservations concerning the practical conclusions that can be drawn from these observations. First of all, the magnitude of the problem in practice and its relevance seems to be considerably smaller than indicated by their study. Actually, the small sample size ($n = 17$) makes these results quite imprecise (the exact 95% CI accompanying 52.9% as derived from "9 out of 17" is 27.8%–77.0%, the lower limit being not far away from our estimate of 27.0% (exact 95% CI: 23.6%–30.6%). Concerning cross-reactivity, concordance beyond chance is very limited considering the whole group of patients. The strong association between the degree of sensitization to colophony and "oakmoss" reactivity, however, could support

the notion of minute traces of resin acids present in "oakmoss" being capable of eliciting a positive reaction at least in patients with extreme sensitivity to colophony. Of course, nonspecific mechanisms like the "angry back syndrome" must also be considered if any very strong reaction is observed, and, indeed (very) strong reactions are generally associated with an increased number of concomitant patch test reactions (J. Brasch, personal communication, 2000).

Last but not least the possibility of concurrent sensitization to "oakmoss" and "treemoss", which are used together in perfumes (Dahlquist and Fregert, 1980) – intentionally or unintentionally (by using "oakmoss" raw material that is often blended with the cheaper "treemoss" material) – had not been taken into account by Lepoittevin *et al* (2000). In principle, highly purified patch test material should be used to standardize patch testing wherever possible – and necessary. The question remains: is pure "oakmoss" patch test material a necessity, in view of the largely combined clinical exposure to "oakmoss" and "treemoss", or, conversely, is not "contaminated" patch test material even more adequate to sensitively diagnose relevant sensitization?

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Reply

To the Editor:

We read with interest the letter from Uter *et al.* (2001) on the concordance between "oakmoss" and colophony in clinical patch testing.

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The aim of our paper (Lepoittevin *et al.* 2000) was to see if patients with a well-established allergy to colophony could react when tested with usual concentrations of "oakmoss" due to the presence of resin acids and their oxidation products. The 17 patients included in our study were therefore selected on the base of their known past and relevant allergy to colophony. The aim of our study was never to evaluate the percentage of concordance between oakmoss and colophony on a large population but to draw attention to a possible misdiagnosis due to the presence of impurities in patch test material.